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Serum Methylmalonic Acid and Total Homocysteine in Patients With Suspected Cobalamin Deficiency: A Clinical Study Based on Gastrointestinal Histopathological Findings

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We compared the sensitivity and specificity of the two metabolite tests, methylmalonic acid (MMA) and total homocysteine (Hcy) in serum, and serum cobalamin (Cbl) in patients referred to our hospital because of suspected cobalamin deficiency and a serum cobalamin value at the referring unit <200 pmol/L. All 111 patients included were investigated using upper gastrointestinal endoscopy with biopsy specimens taken from the gastric and duodenal mucosa to find a morphological basis for cobalamin malabsorption as well as the Schilling test for the validation of the serum tests. All patients were treated with cobalamin and new blood samples were taken after 4 weeks. We found no difference in sensitivity and specificity between serum MMA, Hcy, and Cbl in identifying patients with and without conditions compatible with cobalamin malabsorption. Elevated serum MMA and Hcy were also found in about 15% of the group of patients with normal Schilling tests and without a morphological basis for cobalamin malabsorption. Moreover, most patients in this group responded with decreased values of the metabolite tests following cobalamin treatment, suggesting that neither elevated metabolites nor a decrease in these values following cobalamin treatment are specific for cobalamin deficiency. Am. J. Hematol. 56:230–238, 1997.

Key words: vitamin B_{12} deficiency; methylmalonic acid; homocysteine; diagnostic sensitivity and specificity

INTRODUCTION

Traditionally, cobalamin (vitamin B_{12}) deficiency has been established by the finding of low serum values of cobalamins, often combined with an abnormal Schilling test and/or megaloblastic bone marrow morphology. However, it is now well recognized that cobalamin deficiency sometimes presents as a neurologic or neuropsychiatric disease without concomitant anemia [1]. In the absence of anemia, the diagnosis of deficiency has become increasingly dependent on the determination of serum cobalamins. Unfortunately, the assays now in use have a diagnostic sensitivity and specificity that would be too low to be used as the sole criterion of deficiency [2,3]. The metabolites methylmalonic acid and homocysteine in the two cobalamin-dependent reactions in humans, which accumulate in the serum of deficient patients, have now been introduced as very sensitive tests for cobalamin deficiency [4–7], although the specificity of the tests has been questioned [8,9].

Cobalamin deficiency in non-vegan adults is virtually always caused by malabsorption [10]. In the vast majority of patients, the underlying cause is chronic atrophic gastritis [4,11,12]. Other common causes are previous gastrointestinal surgery. The Schilling test is usually ab-

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normal in small bowel diseases causing cobalamin deficiency such as celiac disease and bacterial overgrowth [13–15]. If symptomless Crohn's disease, in the absence of gut resection, causes cobalamin deficiency, such events are extremely rare [10] and would probably result in an abnormal Schilling test. Impaired cobalamin absorption in pancreatic insufficiency is never of sufficient severity to cause cobalamin deficiency [10].

In the present study, patients prospectively referred because of suspected cobalamin deficiency and with low or low normal concentrations of cobalamins were investigated with regard to the state of the gastrointestinal tract. The results were related to concentrations of serum methylmalonic acid, total homocysteine and cobalamins. The first aim of the investigation was to compare the sensitivity and specificity of serum methylmalonic acid, total homocysteine and cobalamins, respectively, in identifying patients with and without gastrointestinal conditions compatible with cobalamin malabsorption. The second aim was to investigate if the presence of any neutrophil with 6 lobes or more per 100 cells in peripheral blood smears could be a simple alternative to the metabolite tests in identifying patients with cobalamin deficiency.

PATIENTS AND METHODS

Information about the study was distributed to all medical and neurological clinics as well as most private practitioners and primary care centers in the catchment area of Sahlgrenska University Hospital in Göteborg. Patients were recruited by offering an evaluation of all patients with symptoms and signs indicating suspicion of cobalamin deficiency and at least one serum cobalamin concentration value <200 pmol/L. The symptoms and signs that gave rise to the suspicion of cobalamin deficiency were evaluated only by the referring doctors and not by the authors of the study. Of the 163 consecutive patients referred, we included all 111 not yet on substitution treatment, after having excluded one vegan and one patient with uremia. The mean and median serum cobalamin concentrations in the 111 patients were 132 pmol/L (SD 42) and 138 pmol/L, respectively, at the referring units.

All patients underwent upper gastrointestinal endoscopy with a minimum of three biopsy specimens taken from each the gastric body and the second part of duodenum. The gastric body mucosa was histologically classified according to the Sydney system [16] with inflammation and atrophy graded as absent, mild, moderate, or severe. The duodenal mucosa was classified as normal or villous atrophy. The biopsy specimens from each location (duodenum and corpus) were paraffin-embedded and stained with hematoxylin-eosin and PAS (Periodic acid-Schiff). The histological assessment was made on coded sections by the same pathologist (O.N.).

All patients with histologically normal mucosa of the gastric body and the duodenum and no history of gastrointestinal disease or operations interfering with the absorption of cobalamins and normal Schilling tests were classified as ''normals.''

Schilling tests were performed using 1 μg (approximately 20 kBq) ^{57}Co-cyanocobalamin, as a modification of a dual isotope test described by Doscherholmen [17,18] (^{57}Co and ^{58}Co-cyanocobalamin, Amersham International, Buckinghamshire, UK) with the simultaneous measurement of the absorption of ''crystalline,'' i.e., free, and protein-bound cobalamin. Gastric body atrophy with remaining intrinsic factor production is the pathophysiologic basis for ''protein-bound malabsorption.'' We have recently shown that a protein-bound absorption test has poor sensitivity and specificity and that this type of malabsorption is probably better identified with biopsies from the gastric body mucosa showing some degree of gastric body atrophy [18]. We, therefore, have chosen to use only the results from the absorption of free cobalamin in this report. Urinary excretion of >10% of ingested radiolabelled cobalamin was considered normal.

Serum cobalamin concentrations were determined by using a radioassay with purified hog intrinsic factor as the binder (Diagnostic Products Corp., Los Angeles, CA). The coefficient of variation was 15% at 112 pmol/L and 10% at 580 pmol/L. The reference interval for serum cobalamins at our hospital is 130–740 pmol/L. The hospital serves the whole catchment area with these analyses. For the transformation of ng/L, used in other studies, to pmol/L we used pmol/L = ng/L × 0.74.

Folate in whole blood was measured in all cases (Diagnostic Products Corp.). Reference range was 90–450 nmol/L.

Serum methylmalonic acid was determined by a modification of a stable-isotope dilution technique using solid-phase sample extraction and gas chromatography using a mass spectrometer in the selected ion-monitoring mode previously described [19]. The range for methylmalonic acid in 40 healthy volunteers was 0.01–0.4 μmol/L, the mean 0.156 and SD 0.095. Values ⩽0.4 μmol/L were considered normal.

Serum total homocysteine was determined using methods described previously [20–22]. The derivates were separated and quantified using reversed-phase high performance liquid chromatography with fluorometric detection. No significant change in serum homocysteine levels was noted when serum was kept up to 49 hr at ambient temperature, but was noted for blood kept at ambient temperature. The increase in serum homocysteine was 1.4 μmol after 2 hr and 2.4 μmol after 6 hr. The interseries coefficient of variation was 4.9% at 21

TABLE I. Number of Patients With Abnormal Values of Serum Cobalamins, MMA, and Hcy Related to Morphological Findings*

Morphological findings	Serum MMA (upper reference limit 0.4 μmol/L)	Serum Hcy (upper reference limit 13 μmol/L)	Serum cobalamins (lower reference limit 130 pmol/L)
Gastric body atrophy			
Severe (n = 26)	17	13	16
Moderate (n = 6)	2	4	0
Mild (n = 10)[a]	3	3	2
Villous atrophy (n = 6)	3	5[b]	5
Moderate gastric body atrophy + villous atrophy (n = 1)	1	0	1
Body gastritis without atrophy (n = 28)	4	6	8
Normal (n = 34)	6	3	6
Total number of patients with abnormal values	36	34	38

*MMA = serum methylmalonic acid; Hcy = serum total homocysteine.
[a]One of these patients had been subjected to partial gastric resection and had mild atrophy of the remaining gastric mucosa.
[b]Two of these patients had a delayed normalization of Hcy and a concentration of folate in whole blood in the lower reference range.

μmol/L and 9.8% at 9 μmol/L. The mean for homocysteine for 39 healthy men and women aged 20–50 years was 6.25 μmol/L (±2 SD, 6.25 ± 3.94 μmol/L). The upper reference range limit was calculated with correction for the 4–6 hr the blood samples were stored in room temperature with the highest possible increase that is caused by keeping the blood samples uncentrifuged at room temperature up to 6 hr. Thus, as an upper reference limit we have chosen 13 μmol/L. Serum total homocysteine will in this report be designated serum homocysteine.

Peripheral blood smears were evaluated by one of us (BS) for the number of neutrophils with 6 or more lobes in the peripheral blood by counting 100 neutrophils.

Blood specimens for the analysis of serum cobalamins, methylmalonic acid, and homocysteine were drawn on the same day as the endoscopic examination and 4 weeks later. They were centrifuged, frozen, and stored at −20°C. The serum samples from the two visits by each patient were always analysed simultaneously.

The patients received 5 injections, each containing 1 mg of hydroxocobalamin (Behepan®, Kabi Pharmacia, Sweden), intramuscularly within 2 weeks of the initial examination.

Functional cobalamin deficiency was defined as an elevation of serum methylmalonic acid and/or serum homocysteine that normalized after treatment with cobalamin. In two patients, a 40% decrease in homocysteine without reaching the reference range was considered as sufficient.

Statistical Analysis

Student's *t*-test and McNemar's test for paired observations were used.

RESULTS

The mean age of the 111 included patients was 51 years (range 17–80, SD 17, median 50 years) and the mean serum cobalamin concentration at the first visit to our laboratory was 145 pmol/L (SD 54, median 152 pmol/L). Mild anemia was found in nine patients, of whom only two had a hemoglobin value <100 g/L. Both these patients had atrophy of the gastric body. Elevated mean corpuscular volume (MCV > 102 fl) was found in 10 patients although there was a decrease of ⩾5 fl after treatment in only 5.

Sensitivity and Specificity of Serum Cobalamins, Methylmalonic Acid, and Homocysteine in Identifying Patients With a Morphological Basis for Cobalamin Malabsorption

The results of the serum tests in these patients are presented in Table I.

Patients with severe gastric body atrophy. Of the 26 patients with severe gastric body atrophy, 17 (65%) had functional deficiency. There was an agreement in positive results between serum methylmalonic acid and homocysteine in 13 (76%) of these. A corresponding agreement between serum methylmalonic acid and serum cobalamins was found in 14 and between serum homocysteine and serum cobalamins in 12.

All patients with some degree of atrophy of the gastric body mucosa or villous atrophy of the duodenal mucosa. Among the 49 patients in this group, there was an elevation of serum methylmalonic acid in 26, serum homocysteine in 25, and serum cobalamin values below the lower reference limit were found in 24 (Table I). Two of the patients with normal serum methylmalonic acid, el-

evated serum homocysteine, and villous atrophy of the duodenal mucosa did not normalize their homocysteine values at the follow-up 1 month after the first visit to our clinic but on a third visit. They had normal Schilling tests and their whole blood folate concentrations were just above the lower reference limit, suggesting folate dificiency rather than cobalamin deficiency as the cause. Thus, the true number of patients identified with serum homocysteine in this group was probably 23. No significant difference in diagnostic sensitivity was found between any of these tests. There was a co-existing elevation of both methylmalonic acid and homocysteine in 18 of the 33 (55%) patients with functional cobalamin deficiency. Agreement in results between serum methylmalonic acid and cobalamins was found in 19 patients and between serum cobalamins and homocysteine in 16.

The corrrelations between serum cobalamins and serum methylmalonic acid and serum cobalamins and homocysteine are shown in Figure 1a and b.

A comparison of the diagnostic outcome of the three tests in the group of 49 patients with abnormal histology and the 34 patients with normal histology of the gastric body and duodenal mucosa are shown in Table II. There was no statistical difference between the tests in ability to identify these patients.

Patients with gastric body gastritis without atrophy and ''normals.'' In these two groups, comprising 62 patients in all (Table I), with no morphological basis for cobalamin malabsorption and normal Schilling test results, elevated serum methylmalonic acid was found in 10 (16%) and serum homocysteine in 9 (15%) while 14 (23%) had serum cobalamin values below the lower reference limit. There was no significant difference between the tests in the ability to correctly identify the 62 patients.

Mean serum creatinine was higher in the patients with elevated serum methylmalonic acid, 90.1 μmol/L compared to 80.9 μmol/L (reference range 60–120 μmol/L), in those with normal serum methylmalonic acid ($P < 0.05$). No such difference was found for those with elevated serum homocysteine.

Response to Cobalamin Treatment

All patients with elevated serum methylmalonic acid and/or homocysteine (including those with conditions not compatible with cobalamin malabsorption) had a decrease in their initial methylmalonic acid values of at least 40% and in their homocysteine values of at least 25% after treatment with cobalamin. An unexpected finding was the significant lowering of serum methylmalonic acid and homocysteine after cobalamin treatment in the group of 28 patients with gastric body gastritis without atrophy ($P = 0.0001$ and $P = 0.0008$, respectively) and in the group of 33 (one patient was lost in the follow-up) patients with normal mucosa ($P = 0.0001$ and $P = 0.0001$, respectively) (Fig. 2; Tables III and IV). All these patients also had a normal duodenal mucosa and a normal Schilling test result. A corresponding difference in metabolite concentrations in the patients with some degree of atrophy of the gastric body mucosa and normal serum methylmalonic acid and homocysteine measured before and after treatment was also found. The mean difference for methylmalonic acid ($n = 19$) was 0.092 μmol/L ($P = 0.0001$) and for homocysteine ($n = 22$) 1.864 μmol/L ($P = 0.01$).

Correlation Between Serum Cobalamin Values and Functional Deficiency

Using serum methylmalonic acid as an indicator of functional cobalamin deficiency, 7 of the 49 (14%) patients with atrophy of either the gastric body or the duodenal mucosa and cobalamin values within the reference range, displayed deficiency (Fig. 1a). The corresponding figure for homocysteine was 9 (18%) patients (Fig. 1b). Of the 13 patients with a serum cobalamin concentration within the reference range and functional deficiency at the time of our investigation, 7 had at least one value below the lower reference limit taken earlier at the referring unit.

Fourteen of the 111 patients referred with serum cobalamin values <200 pmol/L had values >200 pmol/L on re-examination (range 201–310).

Schilling Test

Seventeen of the 26 (65%) patients with severe, 3 of the 6 (50%) with moderate, and none of the 10 with mild gastric body atrophy had abnormal Schilling tests like 4 of the 6 patients with villous atrophy of the duodenal mucosa. All patients with conditions not expected to give cobalamin malabsorption, i.e., the 28 patients with chronic body gastritis without atrophy and the 34 with normal gastric body mucosa, had normal Schilling test results.

Evaluation of Peripheral Blood Smears

This evaluation was carried out in a sub-population of 57 patients. The results are presented in Table V. There was a poor relationship between the presence of 6-lobed neutrophils and functional deficiency.

DISCUSSION

The present study differs in many respects from previous reports describing the usefulness of serum methylmalonic acid and homocysteine in establishing cobalamin deficiency.

Firstly, we found no difference in ability to identify the 49 patients with gastrointestinal conditions, which can give rise to cobalamin malabsorption, in either of the two metabolite tests and serum cobalamins. Although some caution has to be exercised in the interpretation of these

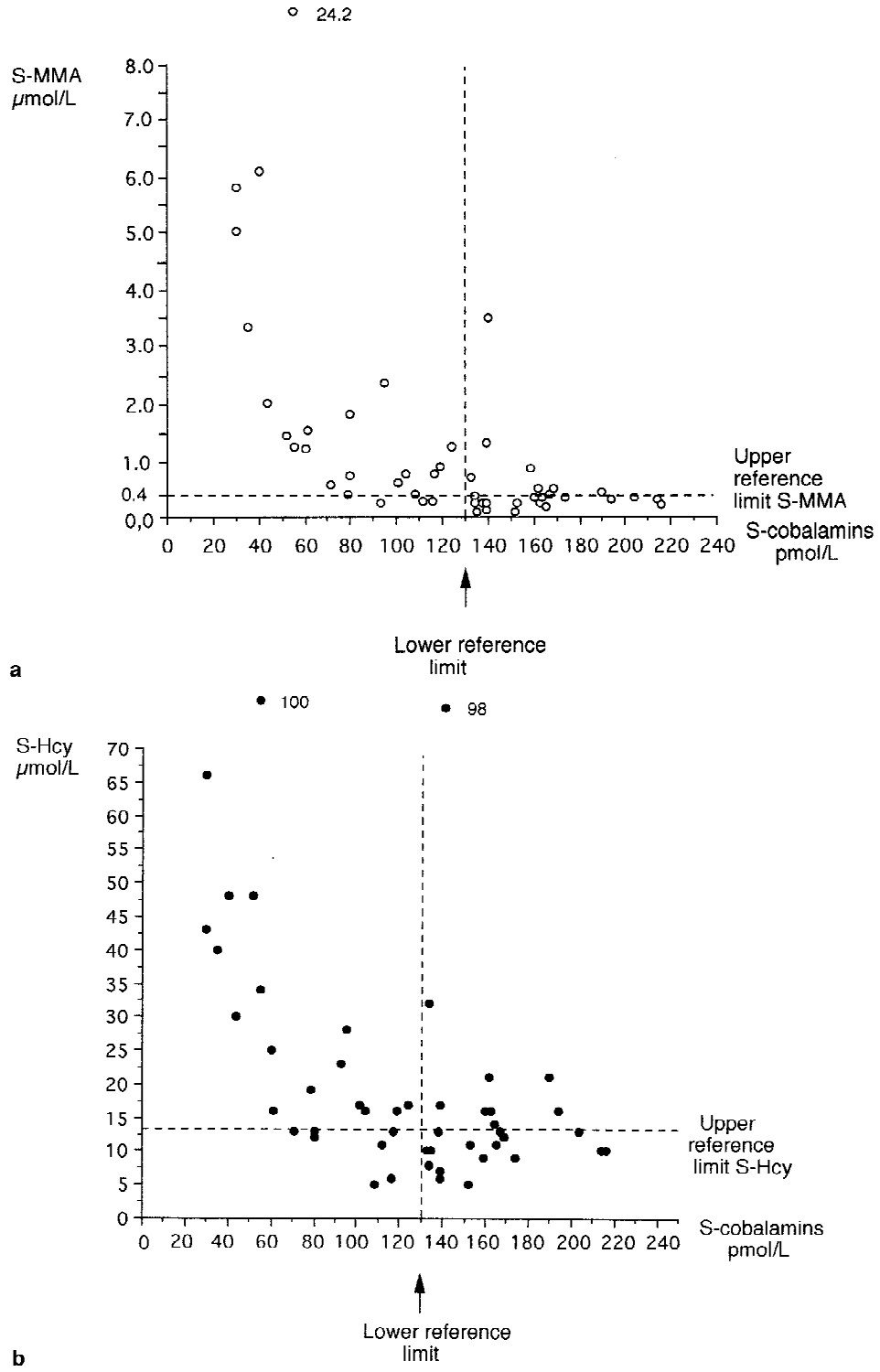

Fig. 1. a: Relationship between the concentration of serum cobalamins and serum methylmalonic acid (MMA) in 49 patients with some degree of atrophy of the gastric body mucosa or villous atrophy of the duodenal mucosa. b: Relationship between the concentration of serum cobalamins and serum homocysteine (Hcy) in 49 patients with some degree of atrophy of the gastric body mucosa or villous atrophy of the duodenal mucosa.

TABLE II. McNemar Plots Comparing the Diagnostic Ability of the Three Tests in 49 Patients With Abnormal Histology and 33 Patients With Normal Histology of the Gastric Body and Duodenal Mucosa and Normal Schilling Test Results*

	Correct	Not correct	
	S-MMA		
S-Cobalamins			
Correct	43	8	$P = 0.81$
Not correct	10	21	
	S-Hcy		
S-Cobalamins			
Correct	40	12	$P = 1.0$
Not correct	13	17	
	S-MMA		
S-Hcy			
Correct	43	10	$P = 0.82$
Not correct	10	19	

*S-MMA = serum methylmalonic acid; S-Hcy = serum total homocysteine.

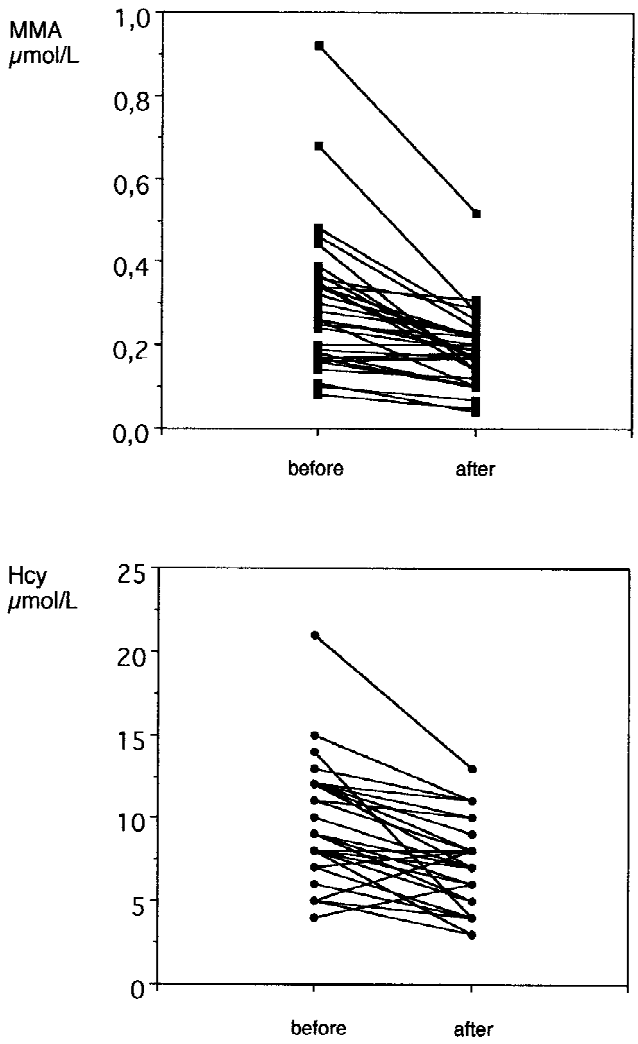

Fig. 2. Values of serum methylmalonic acid (MMA) and serum homocysteine (Hcy) before and after treatment with hydroxocobalamin in 33 patients with normal gastric body and duodenal histology and normal Schilling test results.

results as regards serum cobalamins, since only patients with serum cobalamins <200 pmol/L were included, our findings are in strong contrast with other studies reporting much higher sensitivity for abnormal serum methylmalonic acid and homocysteine concentrations compared to low serum cobalamins [23–25]. Some concern about how to interpret the high prevalence of elevated metabolites in these studies has already emerged [8,9]. None of them has been correlated to signs or symptoms of cobalamin deficiency or to conceivable malabsorptive disorders. Since many other disorders may cause elevated metabolites [26], such conditions might have influenced the results of these studies.

Secondly, elevated serum methylmalonic acid and homocysteine values, as signs of cobalamin deficiency, are usually not expected to be found in patients with normal Schilling tests, normal duodenal mucosa, and absence of atrophy of the gastric body. Increased metabolites in about 15% of this group of patients with each test, calls into question the specificity of the tests as to cobalamin deficiency. These results, in combination with the significant fall in serum methylmalonic acid and homocysteine levels in practically all of these patients after high doses of cobalamin, suggest that elevated metabolites, which normalize after treatment, exist in the absence of cobalamin deficiency. The fall in serum methylmalonic acid after cobalamin treatment in seemingly healthy subjects has also been reported previously [27], although the results were interpreted as mild cobalamin deficiency. Lack of demonstrable malabsorption in our study speaks against such a hypothesis. An intriguing question, however, is why these patients so clearly differ from the healthy volunteers used for establishing the reference range for the tests. The small but significant difference in renal function found between the patients with and without elevated serum methylmalonic acid would probably have been overlooked if the same difference had not been found in the Framingham population [25]. Savage et al. [7] found elevated serum methylmalonic acid in 15 (12.2%) of 123 patients with folate deficiency, of which 14 (11.4%) had renal dysfunction. Correspondingly, the high serum creatinine level for exclusion of patients in the study by Pennypacker et al. [23] (>3.0 mg/dL) might explain the high prevalence of elevated metabolites among these patients. Elevation of serum methylmalonic acid is a constant finding in patients with uremia [28]. Thus, the relationship between renal function and metabolite tests has to be further elucidated before far-reaching conclusions about the prevalence of cobalamin deficiency based on these tests can be drawn.

Thirdly, compared to most previous reports describing the diagnostic usefulness of serum methylmalonic acid

TABLE III. Mean, SD, and Range for Serum Cobalamins (B_{12}), Serum Methylmalonic Acid (MMA), and Homocysteine (Hcy) Before Cobalamin Therapy and Difference Between Values for MMA and Hcy Before and After Cobalamin Therapy in Patients Without Gastric Body Atrophy and With Normal Duodenal Mucosa and Schilling Test Results

Histology group	B_{12} [pmol/L mean (SD), range]	MMA [μmol/L mean (SD), range]	Hcy [μmol/L mean (SD), range]	MMA1-MMA2 (mean difference, μmol/L)	Hcy1-Hcy2 (mean difference, μmol/L)
Chronic body gastritis without atrophy (n = 28)	157 (48) 80–310	0.32 (0.21) 0.09–1.08	10.8 (4.9) 5–29	0.156 (P = 0.0001)	3.464 (P = 0.0008)
Normal histology group (n = 33)	172 (47) 84–275	0.29 (0.17) 0.08–0.92	9.5 (3.5) 4–21	0.109 (P = 0.0001)	2.636 (P = 0.0001)

TABLE IV. Serum Methylmalonic Acid (MMA) and Homocysteine (Hcy) Before and After Cobalamin Treatment and Serum Cobalamins Before Cobalamin Treatment in 33 Patients With Normal Histology of the Gastric Body and Duodenal Mucosa and Normal Schilling Tests*

S-MMA (μmol/L)		S-Hcy (μmol/L)		S-Cobalamins (pmol/L)
Before	After	Before	After	Before
0.92	0.52	8	8	98
0.68	0.28	12	8	164
0.48	0.26	15	11	136
0.46	0.24	13	11	183
0.44	0.14	8	3	92
0.39	0.16	9	7	210
0.37	0.17	12	11	180
0.36	0.29	8	7	255
0.35	0.14	12	10	84
0.34	0.22	9	6	275
0.34	0.17	5	8	112
0.34	0.31	10	7	136
0.32	0.22	12	7	161
0.30	0.22	9	5	162
0.28	0.23	11	10	170
0.26	0.10	14	4	149
0.26	0.20	5	3	228
0.26	0.19	7	8	154
0.25	0.22	12	7	169
0.24	0.19	11	8	172
0.20	0.20	6	4	211
0.19	0.17	7	4	227
0.18	0.10	8	6	161
0.18	0.10	5	4	163
0.17	0.10	11	8	118
0.17	0.17	9	6	183
0.17	0.18	9	6	159
0.16	0.10	21	13	152
0.16	0.17	8	5	181
0.14	0.12	7	4	255
0.11	0.04	12	9	241
0.10	0.07	4	6	144
0.08	0.05	6	4	185

*One patient has been excluded because serum from the follow-up after cobalamin treatment was missing.

and serum homocysteine in establishing cobalamin deficiency, most patients investigated in our study had higher serum cobalamin values. Thus, more than 50% of the patients had values above the lower reference limit. In the study by Lindenbaum et al. [1], 88% had a serum cobalamin value of 111 pmol/L or lower (reference range 148–666 pmol/L) and all patients in Moelby et al.'s study [6] had serum cobalamin values less than 100 pmol/L (reference range 135–585 pmol/L). Furthermore, the validation in our study was not primarily by symptoms or signs of cobalamin deficiency but by gastrointestinal changes causing cobalamin malabsorption. In our view, this makes a better validation possible in early cases of cobalamin deficiency with discrete symptoms and signs. Patients with higher cobalamin values have recently been described [23,25] but in these studies the results have not been supported by signs or symptoms of cobalamin deficiency or validated as to underlying impaired cobalamin absorption. No patient in these studies was under the age of 65 years while the mean and median age in our study was about 50 years. Very few patients in our study had anemia or macrocytosis and no patient had serious neuropsychiatric symptoms.

In the whole group of 49 patients with gastrointestinal pathology compatible with cobalamin malabsorption, agreement in positive outcomes between the two metabolite tests was found in only 55%, reflecting the fact that many of our patients were in an early stage of cobalamin deficiency. In a study reported by Stabler et al. [29] of patients with mild cobalamin deficiency, the corresponding figure was 77%, which corresponds to our figure (76%) for the severe gastric body atrophy group. In contrast, a much higher agreement between the two tests, close to 100%, has been reported in the late stages of deficiency [7]. The explanation for these differences is probably that the depletion of the two co-enzymatically active cobalamins, which regulate the transformation of methylmalonic acid and homocysteine, occurs in one of them before the other.

To find a serum cobalamin level above which functional deficiency is rarely or never seen would be of considerable clinical importance. It is necessary to bear in mind the relatively high coefficient of variation seen in the radioassays in common use. Consequently, a single measurement can show a major deviation from the true level. This is also illustrated by the fact that about half of the patients with a normal cobalamin value and functional deficiency in the blood samples taken at our laboratory had a serum cobalamin value below the lower

TABLE V. Occurrence of 6-Lobed Neutrophils in Peripheral Blood Smears From 57 Patients*

	Patients with atrophy of the gastric body mucosa or villous atrophy of the duodenal mucosa				
No. of 6-lobed neutrophils	Both MMA and Hcy elevated (n = 10)	MMA alone elevated (n = 5)	Hcy alone elevated (n = 6)	Both MMA and Hcy normal (n = 7)	Non-atrophy group (n = 29)
0	3	3	2[a]	5	15
1–2	3	1	3	2	12
⩾3	4	1	1	0	2

*MMA = serum methylmalonic acid; Hcy = serum total homocysteine. Non-atrophy group = patients with normal duodenal mucosa and gastritis without atrophy or normal mucosa of the gastric body.
[a]One of these patients had undergone a partial gastric resection.

reference limit at the referring unit at which only one test for serum cobalamins was taken in the majority of cases. According to our results, functional deficiency can be seen in patients with isolated serum cobalamin values at least up to 200 pmol/L although it is doubtful if such values represent the true level or simply the extreme sum of true biological, temporal, and analytical error variability.

The presence of at least 1% neutrophils with six lobes or more, has been found in 98% of 357 patients with megaloblastic anemia but only in one of the 50 normal controls [30]. In a subgroup of our patients in which peripheral blood smears were examined, the presence of 6-lobed neutrophils seemed to be a very insensitive, nonspecific sign of early cobalamin deficiency. In a recent study, similar results have been reported for neutrophil lobe average and presence of 5 or more lobes [31].

Although the metabolite tests are good supplementary tests in the diagnosis of cobalamin deficiency, the significance of elevated metabolite levels is not yet fully understood. The implication of our data is that if early functional cobalamin deficiency needs to be verified, the simultaneous use of both tests is mandatory. Furthermore, the establishment of cobalamin deficiency should be supported by either typical symptoms or signs of deficiency or the identification of an underlying gastrointestinal disease.

The introduction of the metabolite tests has not fully solved the problem how to diagnose patients with atypical cobalamin deficiency. The next important issue in this field should be to thoroughly elucidate the role of serum holotranscobalamin II, suggested by Herzlich and Herbert [32], as a means for the identification of these patients.

In conclusion, this study confirms that patients with low concentrations of serum cobalamins have increased serum methylmalonic acid and homocysteine values but casts doubt on the interpretation of increases of these metabolites as evidence of cobalamin deficiency in patients with cobalamin values within the reference range. The decrease in serum methylmalonic acid and homocysteine after cobalamin treatment seems to be a common finding and not restricted to patients with gastrointestinal conditions suggestive of cobalamin malabsorption. No difference in diagnostic sensitivity and specificity was found between serum cobalamins, serum methylmalonic acid, and serum homocysteine in identifying patients with a morphological basis for cobalamin malabsorption.

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